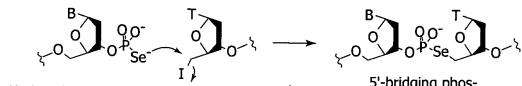
Sheet 1 of 19

Fig. 1a

3'-phosphorothioate 5'-iodothymidine

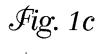
5'-bridging phos-phorothioate linkage

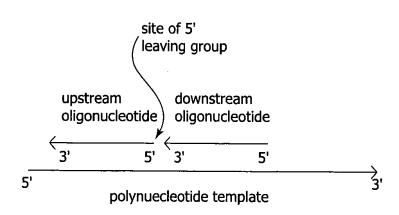
 $\mathcal{F}ig. 1b$



5'-iodothymidine 3'-phosphoroselenoate

5'-bridging phos-phoroselenoate linkage

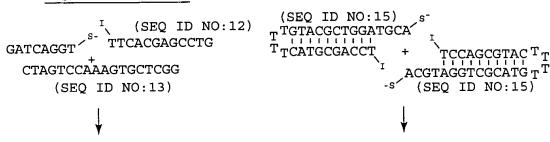




AAFTELA A

ligation of ssDNA

ligation of duplex DNA



 $\begin{array}{c} {\tt GATCAGGT_STTCACGAGCCTG} \\ ({\tt SEQ\ ID\ NO:14}) \end{array}$

TTGTACGCTGGATGCAsTCCAGCGTACTT TTCATGCGACCTsACGTAGGTCGCATGTT (SEQ ID NO:16)

one pot ligation / cyclization of ssDNA

(SEQ ID NO:17) GTTTTATACAAAACCTGGCA

(SEQ ID NO:18) TTCAGCAAAATATGT TTTTGGACCGTTGGT T (SEQ ID NO:19)

CTGCTTCACTAGT TCAGGAGACTGTTCAG

AGTGATCAAGTCCTCTGA (SEQ ID NO:20)

 $_{
m T}^{
m TCAGCAAAATATGT_sTTTGGACCGTTGGT}_{
m T}_{
m C}$ C (SEQ ID NO:21) m TC $m ^T_{
m CTGCTTCACTAGT.TCAGGAGACTGTTCAG}$

Fig. 4a

rxn.type	conversion	isolated yield
ssDNA ligation	>90%	44%
duplex ligation	75%	36%
ligation/cyclization	>90% (1st step) 50% (2nd step)	20%

Fig. 4b

exonuclease/hydrolysis susceptibility

5'-GATCAGGT_{Ps}TTCACGAGCCTG-3' (SEQ ID NO:14)

endonuclease susceptibility

template for replication / transcription

(SEQ ID NO:22)

5'-TAATACGACTCACTATA
3'-ATTATGCTGAGTGATATCCTGCCTATTCCGAGCACTT_{Ps}TGGACTAG
(SEQ ID NO:23)

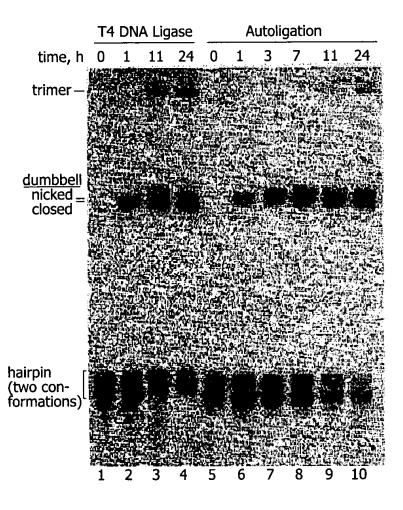


Fig. 6

5'-bridging phosphorothioate duplex: dumbbell—

(SEQ ID NO:24) T^TGTACGCTGGATGCA^S TCCAGCGTAC^TT

T_TCATGCGACCT_sACGT AGGTCGCATG_T

all phosphodiester duplex:

Nsi I

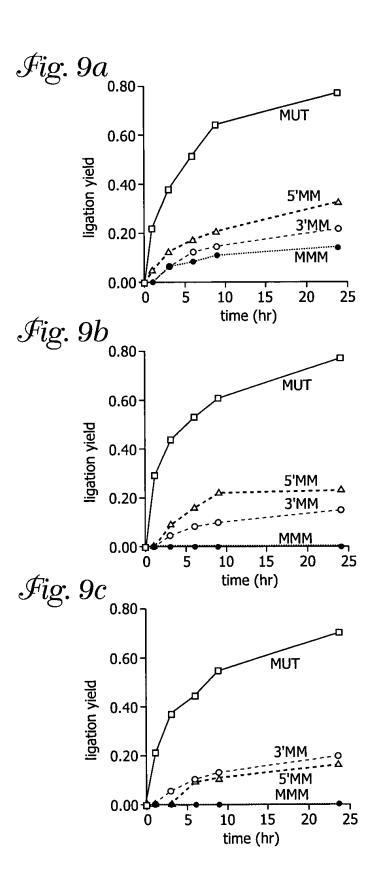
(SEQ ID NO:25) T^TGTACGCTGGATGCA^TCCAGCGTAC^TT

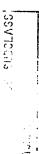
Fig. 7

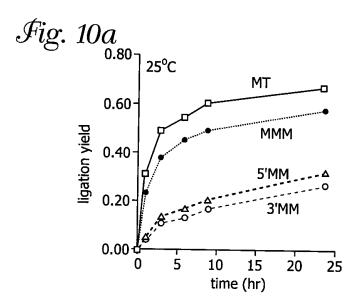
hairpin

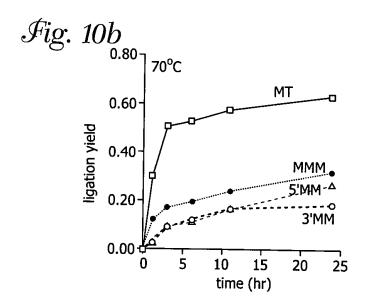
(SEQ ID NO:26) linear probes GTGGGCGCCGPg 1-TCGGTGT 3'CCACCACCGAGGCAGCCACACCCATTC5' (SEQ ID NO:27) MUT target 3'CCACCACCGAGGCÇGCCACACCCATTC5' (SEQ ID NO:28) 5'MM target 3'CCACCACCGAGGAAGCCACACCCATTC5' (SEQ ID NO:29) 3'MM target 3'CCACCACCGAGGCAGGCACACCCATTC5' (SEQ ID NO:30) MMM target $_{\mathrm{T}}$ ACATTAGCACTATAAGCAC $_{\mathrm{T}}$ (SEQ ID NO:31) cyclization probe GTGGGCGCCGP, I-TCGGTGTGGG 3'CCACCACCGAGGCAGCCACACCCATTC5' (SEQ ID NO:27) MUT target 3'CCACCACCGAGGCÇGCCACACCCATTC5' (SEQ ID NO:28) 5'MM target 3'CCACCACCGAGGAAGCCACACCCATTC5' (SEQ ID NO:29) 3'MM target 3'CCACCACCGAGGCAGCCAAACCCATTC5' (SEQ ID NO:32) MMM target











(SEQ ID NO:31) cyclization probe GGCGCCGPs 1-TCGGTGTGGGTTTTC

(SEQ ID NO:33) MUT target 3'ACTACGCGGCAGCCACACCCAAAAGTTC5'

(SEQ ID NO:34) 3'MM target 3'ACTACGCGGAAGCCACACCCAAAAGTTC5'

(SEQ ID NO:35) MMM target 3'ACTACGCTGCAGCCACACCCAAAAGTTC5'

Fig. 11a

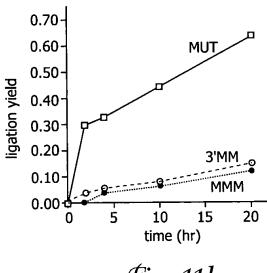


Fig. 11b

Fig. 12a

linear probes

(SEQ ID NO:36)

3'
TGAGAACGGGTGT-1 PsGGCTGCC

(SEQ ID NO:37) WT target

5'GTCAGCGCACTCTTGCCCACACCGCCGG-CGCCCACCACCACCAGCTTATA3'

(SEQ ID NO:38) MUT target

5'GTCAGCGCACTCTTGCCCACACCGACGG-CGCCCACCACCAGCTTATA3'

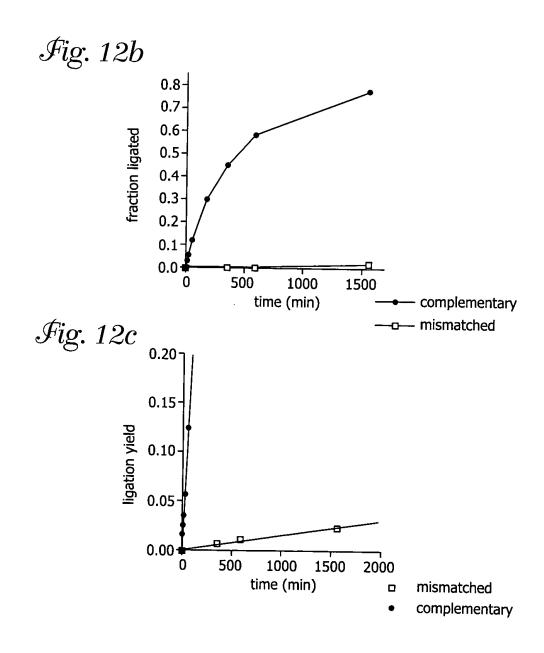
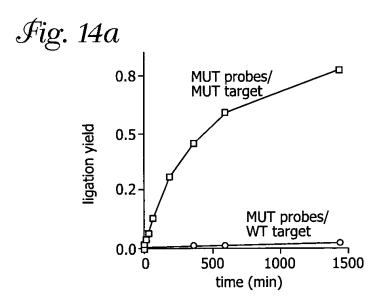
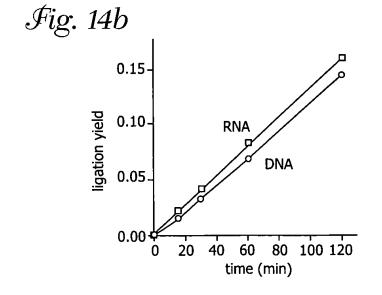


Fig. 13





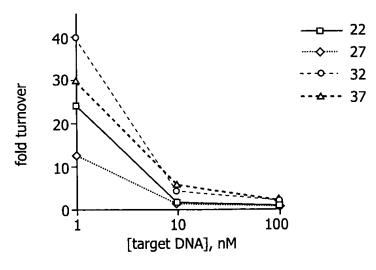
2.2

temperature	[target]	fold turnover	
temperature (°C)	(nM)	no cycling ^a	cycling ^b
22	1	24	
	10	1.6	1.0
	100	1.0	1.0
27	1	13	14
	10	1.6	3.0
	100	1.2	1.2
32	1	40	51
	10	4.6	4.7
	100	2.3	2.3
37	1	30	44
	10	5.9	6.2

 $^{\text{a}}\text{Simple}$ incubation of 10 μM probes with target for 24 hr followed by gel electrophoresis and quantitation of ligated product. $^{\text{b}}\text{24}$ hr of thermal cycling (30 min at temp. shown, followed by 45 sec at 95°C).

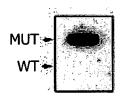
100

Fig. 15a



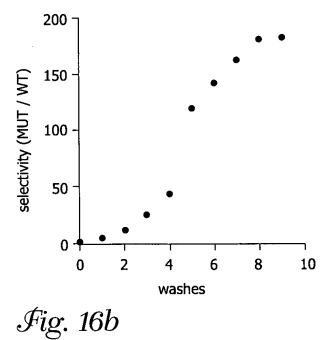
2.2

Fig. 15b

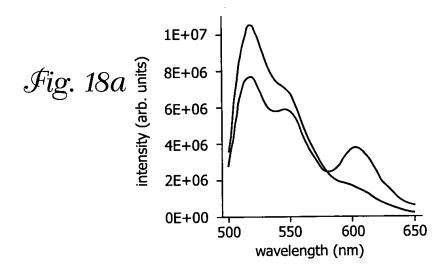


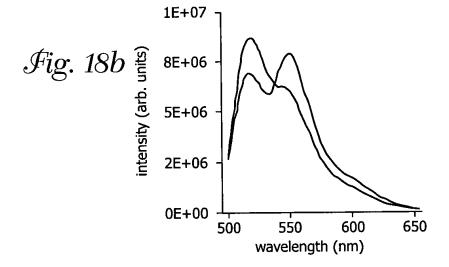
DRAF FOLK

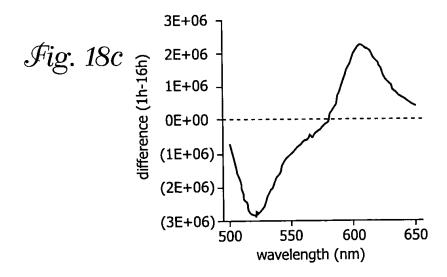
Fig. 16a



universal probe (FAM label)







S. SUBBLASS

Fig. 19

probes (SEQ ID NO:36)

TGAGAACGGGTGT: -seGGCXGCC (X=G,T)

WT target

5'GTCAGCGCACTCTTGCCCACACCGCCGCGCGCCCCACCACCACCAGCTTATA3'
(SEQ ID NO:37)

MUT target (SEQ ID NO:38)
5'GTCAGCGCACTCTTGCCCACACCGACCGCCCCACCACCACCACCACCTTATA3'

MUT RNA target (SEQ ID NO:40)
5'GCGCACUCUUGCCCACACCGACGCGCC3'

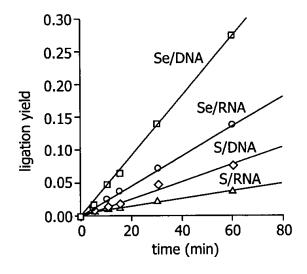


Fig. 20

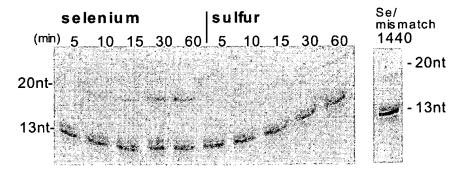


Fig. 21